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Genetic Variance Contributes to Dopamine and Opioid Receptor Antagonist-Induced Inhibition of Intralipid (Fat) Intake in Inbred and Outbred Mouse Strains

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Abstract

Preference for and intake of solid and emulsified fat (Intralipid) solutions vary across different mouse strains. Fat intake in rodents is inhibited by dopamine and opioid receptor antagonists, but any variation in these responses as a function of genetic background is unknown. Therefore, the present study compared the ability of dopamine D1-like (SCH23390) and general opioid (naltrexone) receptor antagonism to alter intake of fat emulsions (Intralipid) in mice. Two-h intakes of 5% Intralipid were measured (5–120 min) in seven inbred (*BALB/c*, *C57BL/6*, *C57BL/10*, *DBA/2*, *SJL*, *SWR*, *129P3*) and one outbred (CD-1) mouse strains following treatment with vehicle, SCH23390 (50–1600 nmol/kg, ip) and naltrexone (0.001–5 mg/kg, sc). SCH23390 significantly, dose-dependently and differentially reduced Intralipid intake at all five (*DBA/2*, *SWR*, CD-1), four (*SJL*, *C57BL/6*), three (*129P3*) and one (*C57BL/10*) of the doses tested, but failed to affect Intralipid intake in *BALB/c* mice. Naltrexone significantly, dose-dependently and differentially reduced Intralipid intake at all four (*DBA/2*), three (*SWR*, *SJL*), two (CD-1, *C57BL/10*) and one (*C57BL/6*, *129P3*) of the doses tested, and also failed to affect Intralipid intake in *BALB/c* mice. SCH23390 and naltrexone were respectively 13.3-fold and 9.3-fold more potent in inhibiting Intralipid intake in the most sensitive (*DBA/2*) relative to the least sensitive (*BALB/c*) mouse strains. A strong positive relationship ($r=0.91$) was observed for the abilities of SCH23390 and naltrexone to inhibit Intralipid intake across strains. These findings indicate that dopaminergic and opioid signaling mechanisms differentially control Intralipid intake across different mouse strains, suggesting important genetic and pharmacological interactions in the short-term control of rewarding and post-ingestive consequences of fat intake.

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Keywords

Dopamine D1 receptor; Opioid receptors; SCH23390; Naltrexone; Fat Intake

1. Introduction

Genetic variance has been observed for many aspects of ingestive behavior in inbred mouse strains (see review: Reed et al., 1997), including the intake of dietary fat (see review: West and York, 1998). Thus, mouse strain differences have been observed in the intake of complete high-fat diets, fat selection in nutrient self-selection paradigms, or intake of fat emulsions (e.g., Alexander et al., 2006; Bachmanov et al., 2001; Glendinning et al., 2008; Sclafani, 2007; Sclafani and Glendinning, 2005; Smith et al., 2000, 2001; Smith-Richards et al., 1999, 2002). Specifically, *AKR* inbred mice consumed moderate amounts of a high-fat diet that promoted weight gain and obesity, whereas carbohydrate-preferring *SWR* mice consumed large amounts of the same diet that was not accompanied by weight gain (Smith et al., 2000, 2001). The different patterns of fat intake and compensatory weight changes observed in *AKR* and *SWR* strains persisted across different types of fat sources and whether the high- and low-fat diets were isocaloric (Smith-Richards et al., 1999). Indeed, whereas fat consumption and accumulation of ependymal fat were correlated in *AKR* and *C57BL/6* mice, fat consumption in *SWR* and *CAST/Ei* strains was inversely correlated with accumulation of ependymal fat (Smith et al., 2000, 2001). Diet-sensitive *AKR* and *DBA/2* strains consumed greater amounts of fat, displayed more adiposity, and displayed elevated levels of leptin and insulin, whereas *C57BL/6* mice showed an equal preference between protein and fat, and displayed normal insulin and leptin levels (Alexander et al., 2006). In contrast, obesity-resistant *SWR* and *A* mice that consumed more fat than carbohydrate displayed lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin, and/or reduced arcuate NPY levels (Leibowitz et al., 2005). Finally, chromosomal mapping analyses revealed a series of genetic loci (mob 1–4), located on chromosomes 9 and 15, that appeared to explain some of these genetic variations for fat and obesity (e.g., Bachmanov et al., 2001; Smith-Richards et al., 2002).

Our laboratory has also investigated genetic variance in fat preference and intake (Lewis et al., 2007) using a liquid fat source in the form of Intralipid, a stable soybean oil emulsion, which is highly attractive and avidly consumed by rodents (e.g., Higgs and Cooper, 1998). Eleven inbred and one outbred mouse strains, including some (*A*, *AKR*, *C57BL/6*, *DBA/2*, *SWR*) described in the earlier studies, were given 24-h two-bottle tests with Intralipid vs. water over a wide range of lipid concentrations (0.0001–5%). We found significant increases in Intralipid intake in *BALB/c* mice at the seven highest concentrations, in *SWR*, *AKR*, *C57BL/6* and *DBA/2* mice at the four highest concentrations, in *CD-1*, *C57BL/10* and *SJL* mice at the three highest concentrations, and in *A*, *C3H/He*, *CBA* and *I29P3* mice at the two highest concentrations, thereby demonstrating a wide degree of genetic variance in the detection sensitivity to consume a fat solution.

Among the multiple pharmacological substrates governing intake of fat in outbred rats are the opioid and dopaminergic receptor systems. Fat intake is significantly reduced in rats by general and selective mu and kappa opioid receptor antagonists (Glass et al., 2000; Higgs and Cooper, 1998; Islam and Bodnar, 1990; Jarosz et al., 2006; Marks-Kaufman et al., 1985; Naleid et al., 2007; Sahr et al., 2008). Chronic mu, mu-1, delta-1, and delta-2 opioid antagonists also significantly decreased weight and intake of a fat source in rats during development of dietary obesity (Cole et al., 1995). Correspondingly, administration of the mu-selective opioid agonist, DAMGO into the nucleus accumbens stimulates high-fat intake in rats (Zhang et al., 1998). Fat intake is also significantly reduced by systemic treatment with dopamine antagonists in

rats as well (Baker et al., 2001; Davis et al., 2006; Rao et al., 2008; Weatherford et al., 1988; 1990).

The aforementioned data therefore indicate that control of a critical macronutrient, fat, is influenced by dopamine and opioid receptor systems and by genetic factors. Whether genetic variance plays a role in the opioid and dopaminergic modulation of fat intake is unknown, and is the purpose of this study. Thus, the present study examined whether genetic variance exists in the dose-dependent ability of general opioid (naltrexone) or dopamine D1-like receptor (SCH23390) antagonism to alter Intralipid intake in a short-term (2 h) test developed previously for the examination of genetic variance in the pharmacology of sucrose intake (Dym et al., 2007, 2009). A 5% Intralipid concentration was selected because it generated substantial intakes in our prior 24-h study (Lewis et al, 2007). We focused on the D1-like antagonist, SCH23390, rather than the D2-like antagonist, raclopride, since the latter drug was relatively ineffective in reducing sucrose intake and/or displaying genetic variance in sucrose intake (Dym et al., 2009). As in our previous studies (Dym et al., 2007, 2009), mouse strains were initially assessed for their ability to display short-term (2 h) Intralipid intake at or above a criterion baseline criterion intake (≥ 1 ml) to allow for the reliable observation of short-acting antagonist-induced reductions. Seven inbred (*BALB/c*, *C57BL/6*, *C57BL/10*, *DBA/2*, *SJL*, *SWR*, *129P3*) and one outbred (*CD-1*) mouse strains met this criterion of short-term Intralipid intake, and were used in the pharmacological analyses, while other strains showing genetic variance in fat intake (*AKR*, *A*) failed to meet this criterion, and were therefore not included.

2. Results

2.1. Strain differences in Intralipid intake following vehicle baseline injections

Evaluation of Intralipid intake following vehicle baseline injections revealed significant differences among strains ($F(7,70) = 7.45$, $P < 0.0001$) and for the interaction between strains and test times ($F(35,350) = 5.68$, $P < 0.0001$). The rank-order of the cumulative 2-h baseline vehicle Intralipid intake among the eight strains was: *BALB/c* (2.2 ml), *CD-1* (2.1 ml), *SWR* (2.0 ml), *DBA/2* (1.7 ml), *C57BL/10* (1.6 ml), *SJL* (1.4 ml), *129P3* (1.4 ml) and *C57BL/6* (1.4 ml). To account for these baseline differences in Intralipid intake, the drug effects were evaluated both on absolute Intralipid intakes and on intakes as a percent of baseline levels.

2.2. Strain differences in SCH23390-induced inhibition of Intralipid intake

Overall significant differences in Intralipid intake following SCH23390 were observed among the eight mouse strains ($F(7,69) = 6.40$, $P < 0.0001$), among doses ($F(5,65) = 51.13$, $P < 0.0001$), across test times ($F(5,65) = 71.36$, $P < 0.0001$), and for all two-way and three-way interactions (P 's < 0.019 - 0.0001). Figure 1 displays the marked strain-specific differences in the dose-dependent and time-dependent ability of SCH23390 to significantly reduce Intralipid intake. *CD-1*, *DBA/2* and *SWR* strains displayed significant reductions across all five SCH23390 doses (Figures 1D, 1E, 1G), whereas *SJL* and *C57BL/6* strains displayed significant reductions following four SCH23390 doses (Figures 1B, 1F). *129P3* and *C57BL/10* strains displayed significant reductions following three and one SCH23390 doses respectively (Figures 1C, 1H). In contrast, *BALB/c* mice failed to display SCH23390-induced reductions in Intralipid intake at any of the tested doses (Figure 1A).

Significant differences in percent baseline values of Intralipid intake following SCH23390 were also observed among strains ($F(7,69) = 9.26$, $P < 0.0001$), among doses ($F(4,66) = 28.20$, $P < 0.0001$), across times ($F(5,65) = 26.81$, $P < 0.0001$), for the strain and time and dose and time interactions (P 's < 0.037 - 0.0001), but not for the interactions between strains and doses or among strains, doses and times. SCH23390 did not alter the percentage of Intralipid intake in *BALB/c* mice at 50, 200 and 400 nmol/kg doses, and only transiently reduced percent intake

by 15% and 35% at the 800 and 1600 nmol/kg doses, respectively. All other strains displayed marked 66–90% inhibition following the 1600 nmol/kg dose, 51–82% inhibition following the 800 nmol/kg dose, and correspondingly dose-dependent lower percentages of inhibition at the three lower SCH23390 doses. The following significant interstrain differences were observed. *BALB/c* mice displayed significantly less inhibition than *DBA/2*, *SWR*, *SJL*, *C57BL/6* and *CD-1* mice following the 50, 400, 800 and 1600 nmol/kg SCH23390 doses, effects that typically persisted across the entire time course. *C57BL/10* mice displayed significantly less inhibition than *DBA/2* mice following the 200, 400 and 800 nmol/kg doses only after 5 min, than *SWR* mice following the 50, 200 and 800 nmol/kg doses only after 5 min, than *SJL* mice following the 50, 200 and 800 nmol/kg doses only after 5 min and the 400 nmol/kg dose after 5–60 min, and than *CD-1* mice following the 200 (5–30 min), 400 (5–120 min) and 800 (5–15, 60–120 min) nmol/kg doses.

2.3. Strain differences in naltrexone-induced inhibition of Intralipid intake

Significant differences in Intralipid intake following naltrexone were observed among the strains ($F(7,68) = 6.07$, $P < 0.0001$), among doses ($F(4,65) = 38.52$, $P < 0.0001$), across times ($F(5,64) = 92.89$, $P < 0.0001$), and for all two-way (P 's < 0.0001), but not the three-way interactions. Figure 2 displays the marked strain-specific differences in the dose-dependent and time-dependent ability of naltrexone to significantly reduce Intralipid intake. Whereas *DBA/2* mice displayed significant reductions across all four naltrexone doses (Figure 2D), *SJL* and *SWR* mice displayed significant reductions following three naltrexone doses (Figures 2F, 2G). The *C57BL/10* and *CD-1* strains displayed significant reductions following the two highest naltrexone doses respectively (Figures 2C, 2D), whereas the *C57BL/6* and *129P3* strains displayed significant reductions only following the highest naltrexone dose (Figures 2B, 2H). In contrast, *BALB/c* mice failed to display naltrexone-induced reductions in Intralipid intake at any of the tested doses (Figure 2A).

Significant differences in percent baseline values of Intralipid intake following naltrexone were also observed among strains ($F(7,68) = 3.60$, $P < 0.002$), among doses ($F(3,66) = 18.89$, $P < 0.0001$), across times ($F(5,64) = 13.65$, $P < 0.0001$), and only for the interaction between strains and times ($F(35,340) = 1.59$, $P < 0.022$), but not for the other two-way or three-way interactions. Naltrexone-induced changes in the percentage of Intralipid intake relative to vehicle was not altered in *BALB/c* mice following the 0.01, 0.1 and 5 mg/kg doses, and was reduced by only 12% following the 1 mg/kg dose. The lowest 0.01 mg/kg naltrexone dose reduced the percentage of Intralipid intake relative to vehicle by 21–41% in only the *CD-1*, *DBA/2*, and *SJL* strains. However, all strains other than the *BALB/c* strain displayed dose-dependent inhibition relative to vehicle of 18–44% following the 0.1 mg/kg dose, 23–59% following the 1 mg/kg dose, and 28–71% following the 5 mg/kg dose. The following significant interstrain differences were observed. *BALB/c* mice displayed significantly less inhibition than *DBA/2* and *SWR* mice following the three highest naltrexone doses, and than *SJL*, *C57BL/6*, *129P3*, *C57BL/10* and *CD-1* mice following the two highest naltrexone doses, effects that typically persisted from 30–120 min. *C57BL/10* mice displayed significantly less inhibition than *DBA/2* mice following the all naltrexone doses only after 5 min, than *SWR* mice following the 1 mg/kg dose for up to 30 min, than *SJL* mice following the 0.01, 0.1 and 5 mg/kg naltrexone doses only after 5 min, than *CD-1* mice following the 0.01 and 5 mg/kg naltrexone doses only after 5 min, and than *129P3* mice following the 0.1 and 1 mg/kg naltrexone doses only after 5 min. *C57BL/6* mice displayed significantly less inhibition than *DBA/2*, *SWR*, *CD-1*, *129P3* and *C57BL/10* mice for up to 30 min following the 1 mg/kg naltrexone dose.

2.4. Strain differences in the ID₅₀ of SCH23390- and naltrexone-induced inhibition of Intralipid intake

Table 1 summarizes the ID₅₀ values for the respective abilities of SCH23390 and naltrexone to inhibit Intralipid intake 60 min following antagonist treatments; these data are consistent with peak inhibition for each time point across the 120 min time course (data not shown) for each strain and each antagonist. *DBA/2* mice displayed the lowest ID₅₀ values for SCH23390-induced (145 nmol/kg) and naltrexone-induced (1.5 mg/kg) inhibition of Intralipid intake. In contrast, *BALB/c* mice displayed the highest ID₅₀ values for SCH23390-induced (1928 nmol/kg) and naltrexone-induced (14 mg/kg) inhibition of Intralipid intake, resulting in respective 13.3-fold and 9.3-fold differences between the most-sensitive (*DBA/2*) and least (*BALB/c*) sensitive strains in D1-like antagonist-induced and general opioid antagonist-induced inhibition of Intralipid intake. There was a strong, significant and positive correlation ($r = 0.91$, $P < 0.01$) between the respective abilities of SCH23390 and naltrexone to inhibit Intralipid intake across strains.

As described in the Discussion section, our laboratory performed parallel studies examining the role of genetic variance in the dose-dependent effects of naltrexone and SCH23390 in inhibiting sucrose intake (Dym et al., 2007, 2009). To examine whether there were any relationships between the inhibitory effects of naltrexone or SCH23390 upon sucrose intake with that of Intralipid intake, Table 1 also provides the ID₅₀ values for the previously-determined (Dym et al., 2007, 2009) respective abilities of SCH23390 and naltrexone to inhibit sucrose intake 60 min following antagonist treatments. For SCH23390, three of the strains (*CD-1*, *DBA/2* and *SWR*) exhibited far greater inhibition of Intralipid intake relative to sucrose intake. In contrast, two strains (*BALB/c* and *129P3*) exhibited far greater D1-like antagonist-induced inhibition of sucrose intake relative to Intralipid intake, whereas the remaining three strains (*C57BL/6*, *C57BL/10* and *SJL*) produced fairly comparable degrees of inhibition following D1-like antagonism. This mixed relationship was confirmed by the lack of a correlation ($r = -0.03$, ns) of SCH23390-induced inhibition of Intralipid and sucrose intake across the tested strains. For naltrexone, four of the strains (*CD-1*, *DBA/2*, *SJL* and *SWR*) exhibited far greater inhibition of Intralipid intake relative to sucrose intake. In contrast, three strains (*BALB/c*, *C57BL/6* and *C57BL/10*) exhibited far greater opioid antagonist-induced inhibition of sucrose intake relative to Intralipid intake, whereas the remaining strain (*129P3*) produced fairly comparable degrees of inhibition following opioid antagonism. This mixed relationship was confirmed by the weak correlation ($r = 0.11$, ns) of naltrexone-induced inhibition of Intralipid and sucrose intake across the tested strains. We (Dym et al., 2009) previously reported that there was a weak correlation ($r = -0.33$, ns) between the respective abilities of SCH23390 and naltrexone to inhibit sucrose intake across the tested strains.

2.5. Heritability estimates of SCH23390- and naltrexone-induced inhibition of Intralipid intake

Table 2 summarizes the narrow-sense heritability estimates for each effective SCH23390 and naltrexone dose at each time point. Whereas relatively consistent heritability estimates ($h^2 = 0.31$ – 0.48) were obtained for baseline vehicle Intralipid intake across the entire 120 min time course (data not shown), a more time-dependent relationship for heritability estimates were obtained across the time course for the different SCH23390 and naltrexone doses. Thus, for SCH23390, heritability estimates were quite modest (e.g., $h^2 = 0.12$) in the first 5 min, and typically increased over time up to the 120 min time point (e.g., $h^2 = 0.42$). This appeared to be associated with the onset of significant SCH23390-induced inhibition of Intralipid intake noted across strains as early as 15 min (*DBA/2*, *SWR*, *129P3*, *CD-1*) and 30 min (*SJL*, *C57BL/6*, *C57BL/10*), but not in *BALB/c* mice (Figure 1). Similarly, with naltrexone, heritability estimates were again modest (e.g., $h^2 = 0.08$ – 0.10) in the first 15 min and time-dependently increased at the 120 min time point (e.g., $h^2 = 0.30$ – 0.42). These effects also appeared to be associated with the onset of significant naltrexone-induced inhibition of Intralipid intake noted

across strains as early as 15 min (*DBA/2*, *SWR*), 30 min (*CD-1*, *SJL*) and 60 min (*C57BL/6*, *C57BL/10*, *129P3*), but not in *BALB/c* mice (Figure 2). Such delays in the onset of naltrexone-induced inhibition of Intralipid intake is consistent with evidence that opioid antagonism is more associated with the inhibition of the maintenance, rather than the initiation of palatable intake (e.g., Frisina & Sclafani, 2002; Kirkham and Cooper, 1988a, 1988b; Rockwood and Reid, 1982).

3. Discussion

Control of a critical macronutrient, fat, is mediated by at least two well-studied factors: a) dopamine and opioid receptor signaling and b) genetic variance. The present study demonstrated that the effectiveness of dopamine D1-like and general opioid antagonists to reduce Intralipid intake was subject to genetic variance. Thus, SCH23390 produced differential and significant strain-dependent reductions in Intralipid intake at five (*DBA/2*, *SWR*, *CD-1*), four (*SJL*, *C57BL/6*), three (*129P3*) and one (*C57BL/10*) of the doses tested, and failed to affect Intralipid intake in *BALB/c* mice. Evaluation of the potency of SCH23390 to produce a 50% inhibition of Intralipid intake revealed a 13.3-fold difference between the most (*DBA/2*) and least sensitive (*BALB/c*) mouse strains. Further, naltrexone produced differential and significant strain-dependent reductions in Intralipid intake at four (*DBA/2*), three (*SWR*, *SJL*), two (*CD-1*, *C57BL/10*) and one (*C57BL/6*, *129P3*) of the doses tested, and also failed to affect Intralipid intake in *BALB/c* mice. Correspondingly, there was a 9.3-fold difference in the potency of naltrexone to produce a 50% inhibition of Intralipid intake in the most (*DBA/2*) and least (*BALB/c*) sensitive strains studied. Interestingly, there was a highly-significant correlation ($r=0.91$) in the strain-dependent abilities of SCH23390 and naltrexone to induce suppressive effects on Intralipid intake across the eight strains. The presence of profound genetic variance in the ability of dopamine D1 and general opioid antagonists to inhibit fat intake is discussed in terms of: a) the importance of genetic variance in differentially mediating the dopaminergic and opioid pharmacological substrates underlying fat and sugar intake; b) their relationship to previous studies demonstrating genetic variance in fat intake and the concept of allelic variation in fat intake; and c) the interacting roles of genetic variance and pharmacological mediation upon fat and sweet intake in relation to food reward.

Genetic Variance and the Pharmacological Substrates Mediating Intralipid and Sucrose Intake

The present study was part of our systematic comparison of the role of genetic variance in sugar (sucrose) and fat (Intralipid) intake, and the role of dopamine and opioid receptor systems in sugar and fat preferences. In comparing genetic variance in 24-h intakes across different concentrations of sucrose (Lewis et al., 2005) and Intralipid (Lewis et al., 2007), twelve mouse strains displayed differences in sucrose (0.0001–20%) intake with the greatest amount of sucrose consumption in *SWR*, *CD-1*, *SJL*, *A*, *C57BL/6*, and *C57BL/10* strains, intermediate intake in *BALB/c* and *129P3* strains, and significantly lower intake in *AKR*, *CBA*, *C3H/He* and *DBA/2* strains. The same 12 mouse strains also displayed differences in Intralipid (0.00001–5%) intake such that significant increases in Intralipid intake were observed in *BALB/c* mice at the seven highest concentrations, in *SWR*, *AKR*, *C57BL/6* and *DBA/2* mice at the four highest concentrations, in *CD-1*, *C57BL/10* and *SJL* mice at the three highest concentrations, and in *A*, *C3H/He*, *CBA* and *129P3* mice at the two highest concentrations (Lewis et al., 2007). Indeed, a positive strong correlation ($r=0.87$) was observed among the 12 strains in the magnitude of sucrose and Intralipid intake, suggesting an underlying close strain-specific relationship between sugar and fat intake.

A second set of studies from our laboratory then examined the role of genetic variance in general opioid (Dym et al., 2007) and dopamine D1-like and D2-like (Dym et al., 2009)

antagonism of sucrose intake. Naltrexone significantly and dose-dependently inhibited sucrose (10%) intake with the greatest sensitivity in *C57BL/10* and *C57BL/6* strains, intermediate sensitivity in *BALB/c*, *C3H/He*, *CD-1* and *DBA/2* mice, and the least sensitivity in *129P3*, *SWR* and *SJL* strains, revealing a 37-fold difference in the ID₅₀ of the most and least responsive strains to naltrexone-induced suppression of sucrose intake (Dym et al., 2007). Raclopride was ineffective in producing strain-dependent effects, and only significantly reduced sucrose intake at the highest test dose in seven of the nine strains. In contrast, SCH23390 significantly reduced sucrose intake at all five doses in *129P3* and *SJL* mice, at four doses in *C57BL/6* and *BALB/c* mice, at three doses in *DBA/2*, *SWR*, *C3H/He* and *C57BL/10* mice, and at the two highest doses in *CD-1* mice, revealing a 3-fold difference in the drug effect between the most and least responsive strains (Dym et al., 2009). A weak and non-significant relationship ($r = -0.32$) was observed between the potency of general opioid and D1-like antagonists to produce a 50% inhibition of sucrose intake across the nine mouse strains.

Given the strong positive correlation among strains between the 24-h intakes of sucrose and Intralipid (Lewis et al., 2005, 2007), we hypothesized that genetic variance in the pharmacological sensitivity to dopamine D1-like and general opioid antagonism in inhibiting Intralipid intake would be similar to the observed genetic variance in dopamine D1-like (Dym et al., 2009) and opioid (Dym et al., 2007) antagonist-induced inhibition of sucrose intake. However, direct comparison of the potencies of SCH23390 and naltrexone to inhibit sucrose and Intralipid intakes failed to reveal similar drug effects. In particular, there were no correlations of SCH23390-induced inhibition of Intralipid and sucrose intake ($r = -0.03$) or naltrexone-induced inhibition of Intralipid and sucrose intake ($r = 0.11$) across the tested strains. Further, our previous studies (Dym et al., 2007, 2009) revealed a weak negative correlation ($r = -0.33$, ns) between the inhibitory effects of SCH23390 and naltrexone on sucrose intake across strains. Further within-strain comparisons of D1-like antagonist effects on the intake of sugar and fat revealed that whereas *CD-1*, *DBA/2* and *SWR* strains exhibited far greater SCH23390-induced inhibition of Intralipid intake relative to sucrose intake, *BALB/c* and *129P3* strains exhibited far greater D1 antagonist-induced inhibition of sucrose intake relative to Intralipid intake. Moreover, whereas *CD-1*, *DBA/2*, *SJL* and *SWR* strains exhibited far greater naltrexone-induced inhibition of Intralipid intake relative to sucrose intake, *BALB/c*, *C57BL/6* and *C57BL/10* strains exhibited far greater opioid antagonist-induced inhibition of sucrose intake relative to Intralipid intake. These data indicate heterogeneous patterns of sucrose and fat intake responses across strains to D1 and opioid antagonism, and a lack of an orderly relationship for rank-ordered genetic variance.

Genetic Variance and Allelic Variation of Fat Intake

The present study revealed a strong relationship between the inhibitory actions of dopamine D1-like and general opioid antagonists on Intralipid intake. In this regard, *DBA/2* mice displayed the most potent inhibition of Intralipid intake following both SCH23390 and naltrexone. *CD-1* mice displayed the second-most potent inhibition of Intralipid intake following both SCH23390 and naltrexone; these were followed by comparable levels of inhibition noted for *SWR* and *SJL* mice. In contrast, *BALB/c* mice failed to display any significant inhibition following any dose of either SCH23390 or naltrexone. Interestingly, changes in heritability estimates obtained across the time course for the different SCH23390 and naltrexone doses appeared to be associated with the onset of significant SCH23390-induced inhibition of Intralipid intake noted across strains as early as 15–30 min as well as the delayed onset of significant naltrexone-induced inhibition of Intralipid intake.

Three strains (*SWR*, *DBA/2* and *C57BL/6*) examined in the present study have considerable previous information concerning their responsiveness to fat. Although generally considered a carbohydrate-preferring strain, *SWR* mice consumed large amounts of a fat diet that was not

accompanied by weight gain, and was inversely correlated with accumulation of ependymal fat (Smith et al., 2000, 2001). These effects persisted in *SWR* mice irrespective of the make-up of the fat source and whether the high- and low-fat diets were isocaloric (Smith-Richards et al., 1999). Potential mechanisms by which *SWR* mice consumed more fat than carbohydrate, yet failed to gain weight, were lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin, and/or reduced arcuate NPY levels (Leibowitz et al., 2005). *DBA/2* strains consumed greater amounts of fat, displayed more adiposity, and displayed elevated levels of leptin and insulin (Alexander et al., 2006). *C57BL/6* mice self-selected the highest proportion of fat in macronutrient diet selection with accumulation of ependymal fat correlating with fat consumption (Smith et al., 2000, 2001). Further, the *C57BL/6* strain showed an equal preference between protein and fat, and displayed normal insulin and leptin levels (Alexander et al., 2006). Thus, *DBA/2* and *C57BL/6* strains act like *AKR* mice in their avidity for fat that correlated with weight gain, whereas *SWR* mice consumed fat, but failed to show weight gain. The dopaminergic and opioid pharmacological controls of Intralipid intake do not show this dichotomous relationship among these strains. Whereas *DBA/2* mice exhibit the greatest sensitivity to dopamine D1-like and opioid antagonism of Intralipid intake, *SWR* mice are nearly as sensitive to SCH23390-induced inhibition of Intralipid intake as the *DBA/2* strain and almost 3-fold more sensitive relative to the *C57BL/6* strain. For opioid antagonism, *C57BL/6* and *SWR* mice display comparable sensitivity that is 2.5-3-fold less sensitive than *DBA/2* mice. Two of the three other strains previously showing genetic variance in fat intake (*AKR*, *A*) failed to meet the criterion of a minimum consumption of at least 1 ml of Intralipid in the short-term (2 h) intake paradigm, and were therefore not included in the pharmacological analysis. In sum, it does not appear that dopaminergic or opioid signaling contribute to these previously described strain-dependent dichotomies between fat intake and weight accumulation.

Another area of allelic variation especially when considering opioid control of Intralipid intake as a function of mouse strain is baseline differences in opioid receptor mRNA and endogenous opioids. The *C57BL/6* and *DBA/2* strains have been examined because of their respective preference and avoidance responses for alcohol that are under opioid control. *DBA/2* mice have higher contents of kappa opioid receptors, pro-dynorphin mRNA and dynorphin peptides in the limbic system relative to *C57BL/6* mice (Jamensky and Gianoulakis, 1997; Korostynski et al., 2006; Winkler and Spanagel, 1998). Further, alcohol-preferring *C57BL/6* and alcohol-avoiding *BALB/c* mice also show differential kappa opioid receptor splicing and gene expression, whereas *DBA/2* differ from both of these strains in yet another aspect of kappa receptor gene expression (Saito et al., 2003). In contrast, *CXBK* mice have decreased delta opioid receptor binding than their *BALB/c* progenitors (Kest et al., 1998), but similar mu and delta binding relative to outbred Swiss-Webster mice (Duttaroy et al., 1999; Duttaroy and Yoburn, 2000). It should be noted that the present study employed naltrexone, a general opioid antagonist with actions at mu, delta and kappa opioid binding sites. Future studies using selective mu, delta and kappa opioid receptor subtype antagonists might amplify this genetic variation further.

Perhaps the greatest example of genetic or allelic variation in the pharmacological substrates of fat intake is the *BALB/c* strain which failed to display any inhibition of Intralipid intake even at very high doses of SCH23390 or naltrexone. One possible reason for this is the avid fat appetite of *BALB/c* mice as indicated by their high short-term (2 h) vehicle baseline intake as well as their strong 24 h preference for Intralipid over water at seven different concentrations (0.001–5%) (Lewis et al., 2007). In contrast, strains (*DBA/2*, *SWR*, *C57BL/6*) sensitive to dopamine and opioid antagonism showed increased Intralipid intake over water intake only at the four highest concentrations (0.5–5%) (Lewis et al., 2007). South and Huang (2006) examined five strains for preferences between high-fat/low-carbohydrate and low-fat/high-carbohydrate diets and found that *C57BL/6* mice consumed 72% of their calories as high fat,

whereas *BALB/c* mice failed to display a preference. *BALB/c* mice showed a 34% higher expression level of agouti-related peptide, but not proopiomelanocortin than *C57BL/6* mice, suggesting important strain differences in this feeding-related peptide.

The present findings can also be considered in light of emerging quantitative trait loci (QTL) analyses of fat intake and its consequences. An initial study (Smith-Richards et al., 2002) examined a F2 population derived from fat-preferring (*C57BL/6*) and carbohydrate-preferring (*CAST/Ei*) strains that were given a choice of either a fat/protein or carbohydrate/protein diet. QTL analyses revealed three significant loci for fat intake on Chromosomes 8 (Mnif1), 18 (Mnif2) and X (Mnif3). A thrifty gene on Chromosome 14 defined by caloric efficiency was a dominant-acting genetic component of high-fat diet-induced obesity in a F2 intercross of *C57BL/6* and *129S6/SvEv* mice (Almind and Kahn, 2004). Finally, using a F2 intercross, QTL analyses of fat depots in mouse lines divergently selected for food intake revealed that Chromosomes 4 and 19 were associated with both white and brown adipose tissue depots, whereas Chromosome 9 was associated with only white adipose tissue depots (Rance et al., 2007). It would be of future interest to link underlying pharmacological control of fat intake with QTL analyses of highly-sensitive (e.g., *SWR*, *DBA/2*) and less-sensitive (*BALB/c*) strains.

Roles of Genetic Variance and Pharmacological Substrates in Fat and Sugar Intake Pertaining to Food Reward

The present and previous (Dym et al., 2007, 2009) findings are of more general interest because dopamine and opioid receptors are thought to mediate different aspects of food reward (Berridge, 1996; Barbano & Cador, 2007). In particular, Berridge (1996) initially proposed that dopamine receptors mediate food ‘wanting’ whereas opioid receptors mediate food ‘liking’ although he more recently identified an opioid ‘wanting’ system as well (Berridge, 2009). Although there are exceptions (Berridge, 1996, 2009), food wanting and liking are interrelated. An implicit assumption is that such mechanisms are centrally-mediated. However, the present study employed a systemic route of drug administration, raising the possibility that the pharmacological effects of treatments may occur outside the central nervous system. The fact that SCH23390-induced and naltrexone-induced suppressive effects on Intralipid intake were highly correlated ($r = 0.91$) in the eight strains, would appear to suggest a close relationship between the food liking and wanting systems among the mouse strains tested, at least for a liquid fat source. Of interest is the possibility that the differential effects of naltrexone and SCH23390 on sucrose and Intralipid intakes are related to neurotransmitter-specific effects on nutrient intake and preference. Several macronutrient diet selection studies suggest that brain opioid receptors are specifically involved in fat preference, although other studies suggest that opioids modulate the intake of preferred foods irrespective of nutrient content (Glass et al. 1999). There is less information available on the possible nutrient specificity of dopamine drug effects. An important consideration in evaluating claims of neurotransmitter-nutrient specificity, are findings that drug effects may be influenced by the specific nutrient type used (e.g., sugar vs. starch, corn oil vs. lard; Glass et al., 1999). The physical form of nutrients (e.g., liquid vs. solid) may also influence drug effects since it can have profound effects on food preference and consumption (Lucas et al., 1989; Sclafani, 1987). Therefore, it is possible that the present results obtained with sucrose and Intralipid solutions would not be replicated in mice offered solid diets or with different carbohydrate and lipid sources. These considerations aside, it is clear that intake of solutions rich in sugar and fat are differentially inhibited by dopamine D1-like and general opioid antagonists in different inbred strains of mice, thereby demonstrating an interaction between genetic variance and pharmacological mechanisms of action.

4. Experimental Procedure

4.1. Subjects

Outbred (CD-1, Charles River Laboratories, Wilmington, MA; $n=11$) and seven inbred *BALB/cJ*, *C57BL/6J*, *C57BL/10J*, *DBA/2J*, *SJL/J*, *SWR/J* and *129P3/J* (Jackson Laboratories, Bar Harbor, ME; $n=10-12$) male mice (6 weeks of age) were initially acclimated to the Queens College vivarium for 1 week in group housing (5 per cage). The animals were then housed individually in plastic cages ($30 \times 20 \times 15$ cm) with stainless steel tops and maintained on a 12 h light/12 h dark cycle (lights off at 2000 h) at a constant temperature of 22°C. All animals were provided with chow (Lab Diet Mouse Chow 5015) and water *ad libitum* throughout the experiment, except when experimental testing was conducted. It should be noted that the fat content of the mouse chow is higher than standard rodent chow (e.g., 26% by energy) which may influence fat preference (Reed and Friedman, 1990). However, the same chow diet was used in our prior studies of sucrose and fat intake. All procedures were approved by the Queens College Institutional Animal Care and Use Committee.

4.2. Intralipid intake procedure

At 3–7 h into the light cycle, chow and water were removed from the cage, and each animal was given 2-h access to 5% Intralipid. The Intralipid was prepared by diluting 20% Intralipid (Baxter Healthcare Corporation, Deerfield, Illinois) with water and was presented in a 10 ml plastic syringe (8 ml Intralipid solution and 2 ml air to allow robust licking behavior) fitted with a stainless steel sipper tube (Dym et al., 2009). Intakes were measured to the nearest 0.2 ml at 5, 15, 30, 60, 90, and 120 min and then food and water were returned. Each animal was exposed to one Intralipid session per day until a criterion minimum of 1 ml was consumed over three consecutive exposures; three to five sessions were typically needed to reach this criterion in tests conducted approximately three times each week; the eight strains listed above met this criterion. A ninth strain (*C3H/He*) that was included in prior studies of opioid and dopaminergic antagonist actions on sucrose intake (Dym et al., 2007, 2009), failed to reach criterion and was excluded. This criterion was employed to avoid “floor effects” of antagonist treatment. Following this initial training period, the mice received an intraperitoneal (ip) vehicle injection (0.3 ml distilled water/30 g body weight, 10 ml/kg) and Intralipid intake was measured over 2 hr. Intralipid tests were then conducted following ip injection of the D1 dopamine receptor antagonist (SCH23390, Sigma Chemical Co., St Louis, MO) at doses of 50, 200, 400, 800 and 1600 nmol/kg and subcutaneous injection of the opioid receptor antagonist (naltrexone, Sigma Chemical Co.) at doses of 0.01, 0.1, 1.0 and 5.0 mg/kg. These dose ranges were identical to those used in strain surveys of D1 and opioid antagonist effects upon sucrose intake (Dym et al., 2007, 2009). Subgroups of animals of each strain, matched for vehicle Intralipid intake, were tested with one of four drug regimens: ascending series of SCH23390 doses followed by a descending series of naltrexone doses, descending series of SCH23390 doses followed by an ascending series of naltrexone doses, an ascending series of naltrexone doses followed by a descending series of SCH23390 doses, or descending series of naltrexone doses followed by an ascending series of SCH23390 doses. A minimum of 72 h elapsed between injections to minimize carry-over effects as performed in previous pharmacological studies on sucrose intake (Dym et al., 2007, 2009). Indeed, orders of drug or specific dose presentation failed to produce any significant effects, insuring lack of order or carry-over effects. Following all antagonist treatments, Intralipid intake was reassessed following a second vehicle injection. Analyses of Intralipid intake under the two vehicle treatments failed to reveal any differences, and therefore, these data were averaged and served as the vehicle baseline. Sample size differences among strains reflect deaths of individual animals or a failure to reach criterion intake for a given animal.

4.4. Statistics

In assessing changes in cumulative Intralipid intake across strains, a three-way randomized block analysis of variance was performed with the nine strains as a between-subjects variable, the pooled vehicle and SCH23390 or naltrexone doses as one within-subject variable, and the six test times as the second within-subject variable. Bonferroni comparisons ($P < 0.05$) evaluated significant drug effects within groups. Because the mouse strains differed in their 2 hr baseline Intralipid intakes, Intralipid intakes following drug treatment were expressed as percent intakes ($\text{intake}/\text{baseline intake} \times 100$) and were evaluated by a three-way analysis of variance as above. The effects of SCH23390 or naltrexone across doses and times upon cumulative Intralipid intake were also evaluated within each strain using two-way repeated-measures analyses of variance with the pooled vehicle and SCH23390 or naltrexone doses as one within-subject variable, and the six test times as the second within-subject variable. Post-drug intake difference scores were also calculated by subtracting Intralipid intake following each SCH23390 or naltrexone dose condition from pooled baseline intake for each animal in each strain. Then linear regression analyses were performed for each time point for each strain with SCH23390 or naltrexone dose as the independent variable and the difference scores for each mouse in each strain as the dependent variable to determine the dose that would inhibit Intralipid intake by 50% (ID_{50}). Finally, narrow-sense trait heritability was determined by comparing the between-strain sum of squares to the total sum of squares for each cumulative intake score for vehicle and each effective dose of SCH23390 or naltrexone at each time point after drug administration. Because animals are isogenic (i.e., genetically identical) within individual inbred strains, between-strain variance (measured by the sum of squares between-subjects score) provides a measure of additive genetic ('allelic') variation (VA), whereas within-strain variance ('error variance', measured by the sum of squares error score) represents environmental variability (VE). An estimate of narrow-sense heritability (h^2) for each trait was obtained using the formula: $h^2 = VA/(VA+VE)$.

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Figure 1.

Alterations (mean, $\pm$ SEM) in Intralipid intake following five doses of the D1-like dopamine receptor antagonist, SCH23390 in eight mouse strains. Individual analyses of variance revealed significant main dose effects and interactions between doses and times for all, but the *BALB/c* mouse strains. Significant ($P < 0.05$) decreases (*) in Intralipid intake following specific drug doses at particular time points relative to corresponding vehicle intake are denoted.

Figure 2.

Alterations (mean, $\pm$ SEM) in Intralipid intake following four doses of the opioid receptor antagonist, naltrexone in eight mouse strains. Individual analyses of variance revealed significant main dose effects and interactions between doses and times for all, but the *BALB/c* mouse strains. Significant ($P < 0.05$) decreases (*) in Intralipid intake following specific drug doses at particular time points relative to corresponding vehicle intake are denoted.

TABLE 1

Linear regression values (ID₅₀) of SCH23390 (SCH: nmol/kg)- and naltrexone (NTX: mg/kg)-induced inhibition of Intralipid (FAT; 5%) and sucrose^{a,b} (SUC; 10%) intakes among the inbred and outbred mouse strains at 60 min.

Strain	SCH FAT	SCH SUC ^b	SCH Ratio	NTX FAT	NTX SUC ^a	NTX Ratio
BALB/c	1928	890	2.2	14.0	4.6	3.0
C57BL/6	598	694	0.9	4.4	1.5	2.9
C57BL/10	1112	998	1.1	4.3	0.5	8.6
CD-1	174	1059	0.2	2.6	5.4	0.5
DBA/2	145	681	0.2	1.5	4.7	0.3
S/L	487	729	0.7	3.5	7.6	0.5
SWR	203	964	0.2	4.0	8.8	0.5
129P3	1016	513	2.0	8.4	10.0	0.8
Sensitivity Ratio ^c	13.3 ^d	2.1 ^e		9.3 ^f	20 ^g	

^aData derived from Dym et al., 2007

^bData derived from Dym et al., 2009

^cThe sensitivity ratio refers to the potency shift of antagonist-induced inhibition of intake (ID₅₀) between the least sensitive strain on a particular antagonist and intake measure relative to the most sensitive strain.

^dSCH FAT: BALB/c vs. DBA/2

^eSCH SUC: CD-1 vs. 129P3

^fNTX FAT: BALB/c vs. DBA/2

^gNTX SUC: 129P3 vs. C57BL/10

Heritability estimates for percent baseline vehicle intake scores across strains for each SCH23390 and naltrexone dose at each time point.

TABLE 2

Time → Condition ↓	5 min	15 min	30 min	60 min	90 min	120 min
SCH23390						
50 nmol/kg	0.23	0.20	0.22	0.20	0.30	0.32
200 nmol/kg	0.18	0.18	0.24	0.20	0.27	0.22
400 nmol/kg	0.31	0.31	0.32	0.37	0.37	0.41
800 nmol/kg	0.12	0.19	0.30	0.28	0.30	0.42
1600nmol/kg	0.20	0.25	0.26	0.23	0.26	0.25
Naltrexone						
0.01 mg/kg	0.20	0.28	0.22	0.23	0.35	0.37
0.1 mg/kg	0.10	0.08	0.13	0.22	0.36	0.33
1.0 mg/kg	0.09	0.13	0.17	0.20	0.25	0.30
5.0 mg/kg	0.35	0.38	0.34	0.40	0.46	0.42